

INTERACTION WITH IODINE METABOLISM FOLLOWING INGESTION OF *ALLIUM CEPA* AND *ALLIUM SATIVUM* BY ALBINO WISTAR RATS

ABSTRACT

The interaction with iodine metabolism following ingestion of *Allium cepa* (onion) and *Allium sativum* (garlic) by albino Wistar rats have been studied. The determination of the effect of the oral administration of onion and garlic extract on thyroid hormones (triiodothyronine, T_3 , thyroxine, T_4) were studied. The mean values of triiodothyronine (T_3) for the experimental animals were 1.23 ± 0.09 ng/ml for the control group and 0.97 ± 0.13 ng/ml for iodine treated high dose garlic group. Compared to the control, administration of onion and garlic led to a statistically significant ($P < 0.05$) decrease in T_3 of both normal and iodine treated rats. The mean values of thyroxine, T_4 , for the experimental animals was found to be 1.19 ± 0.12 IU/ml and 0.85 ± 0.16 IU/ml for the low dose onion normal rats and low dose garlic normal rats respectively. Except for the groups administered with low dose onion, oral administration of onion and garlic extract led to a significant ($P < 0.05$) decrease in the thyroxine level of both normal and iodine treated rats. From these results, it can be concluded that oral administration of onion and garlic led to a significant depression in the serum levels of the two thyroid hormones T_3 and T_4 . It is therefore suggested that these spices should be consumed with caution, especially in at risk of iodine deficiency.

KEY WORDS: Iodine metabolism, *Allium cepa*(onion), *Allium sativum*(garlic)

INTRODUCTION: Micronutrients are substances (organic and inorganic) required in small quantities for a variety of biochemical functions and which, generally, cannot be synthesized by the body and must therefore be supplied in the diet. These include iodine, iron, zinc and vitamin A amongst others. Micronutrients enhance the nutritional value of food and have a profound impact on a child's development and a mother's health.

The thyroid gland is unique among the endocrine glands for its dependence on the essential micronutrient, iodine, for normal hormone production. The thyroid gland actively accumulates and traps iodide from the bloodstream to support its hormone synthesis. The thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) are synthesized from the amino acid, tyrosine

and iodine. These hormones help regulate metabolic rate and promote growth and development throughout the body, including the brain.

Therefore, iodine deficiency is the world's most prevalent, yet preventable cause of brain damage (WHO,2011). The thyroid gland is unique among the endocrine glands for its dependence on this essential micronutrient, iodine, for normal hormone production. The thyroid gland actively accumulates and traps iodide from the bloodstream to support its hormone synthesis .The thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) are synthesized from the amino acid, tyrosine and iodine. The role of dietary iodine deficiency as the principal environmental determinant in the development of endemic goitre and other iodine deficiency diseases (IDD) is firmly established. However, there is epidemiological and experimental evidence that concurrent exposure to naturally – occurring thyroid antimetabolites magnifies the goitre endemia and may also enhance the expression of other related disorders. Another evidence is the fact that iodine supplementation may not always result in complete eradication of goitre, there have been some well-defined instances of geographical pockets of persistence where the condition remains endemic. Sulphurated organic compounds, e.g. thioglucosides, thiocyanate, aliphatic disulphides; phenolic compounds e.g. resorcinol and substituted resorcinols such as m-dihydroxy acetophenone flavonoids or polyhydroxyphenols e.g. tannins, phloretin are some of the various classes of environmental compounds known or suspected to be responsible for the persistence of goitre. These agents are either naturally occurring in staple foodstuffs and natural domestic water supplies or as pollutants resulting from industrial and environmental pollution of water supplies and farmlands. Infact, according to Ubom (1991) dietary iodine absorption and incorporation is reduced by smoking, thiocynates, isothiocynates, nitrates, florides, calcium, magnesium, iron in food and water.

Onion (*Allium cepa*), garlic (*Allium sativum*) and other *Alliums* are important because of the culinary value of their flavours, odours, and also their “mystical healing powers”. Presently, little is known about the effect of some of the phytochemical components of these *Alliums* on biochemical processes especially iodine metabolism and consequently the activity of the thyroid gland. It has been suspected that the aliphatic disulphide abundant in the *Allium* family may cause diets which originally are adequate in iodine to become iodine deficient and these components may also directly affect the uptake of the element by the thyroid, and hence altered levels of T₃ and T₄ hormones. Iodine deficiency is an issue that has attracted global concern and World Health Organisation (WHO) and other allied health organizations towards the close of the last millennium put in place strategies aimed addressing the problem of iodine deficiency. The ingestion of aliphatic disulphides presently in the *Alliums* (particularly onion and garlic) even in diets or in phytotherapy may limit the efforts at the eradication of iodine deficiency. It is on the basis of this that a clear picture of the effect of the consumption or exposure to garlic and onion rich diets on biochemical processes and in particular iodine metabolism is desired. Based on the afore mentioned, the present study is designed to assess the effect of *Allium cepa* and *Allium sativum* on the thyroid hormone profile of normal wistar albino rats and wistar albino rats exposed to iodized salts.

METHODOLOGY

The onion and garlic sample used for this study were purchased from Ika Ika Qua Market in Calabar, capital city of Cross River State of Nigeria.

The dry scaly part of the onion bulbs were removed and the fresh bulbs were properly washed. A quantity of 300g of the onions were weighed and macerated in 300ml of deionized water using an electric blender. The homogenous mixture obtained after maceration was filtered

through a chesse cloth and the residue removed, dried and weighed. The solution left behind weighing 455.7g was used as whole onion extract and from this stock; high and low doses were obtained for the experiments

The dry scaly outer part of the garlic cloves was removed and the fresh cloves were properly washed. A quantity 300g of the garlic were weighed and macerated in 300ml of deionized water using an electric blender. The solution left behind was used as whole garlic extract of 455.7g was used as whole garlic extract and from this, high and low doses were obtained for the experiment.

A quantity of 400mg of potassium iodide were dissolved in 400mls of water. 0.8ml of the solution corresponding 0.8mg/kg body wt was administered to the animals.

Experimental animals

A total of 100 albino rats of the Wistar strain consisting of both male and females were obtained from the disease-free stock of the departmental animal house of biochemistry department, faculty of Basic Medical sciences, University of Calabar, Nigeria. These animals weighing between 80g-120g were used for the experiment.

The animals were housed in Perspex cage, (North Kent Plastic Cages Ltd, England) with bottom grid and a stainless steel top. The animals were kept under adequate ventilation at temperature and relative humidity of $26\pm 2^{\circ}\text{C}$ and 46% respectively. Feed and water were provided *ad libitum*. There weights were taken 3 times during the course of the 14 days.

Animal grouping and treatment

The animals were randomly allocated into ten study groups of ten animals each based on their average weight and litter origin. The groups were treated as stated below:

Group 1	-	control – No treatment(placebo)
Group 2	-	positive control – potassium iodide solution only
Group 3	-	low dose of garlic only
Group 4	-	high dose of garlic only
Group 5	-	low dose of onion only
Group 6	-	high dose of onion only
Group 7	-	low dose of garlic + potassium iodide solution
Group 8	-	high dose of garlic + potassium iodide solution
Group 9	-	low dose of onion + potassium iodide solution
Group 10	-	high dose of onion + potassium iodide solution

Administration of the extracts

The administration of the aqueous extracts to the different groups of animals was done for 14 days. 1ml of both the onion and the garlic extracts containing 1.14g of each sample of onion and garlic extract was designated as low dose while 1.5ml containing 1.70g of each sample was designated as high dose.

About 0.8mg/kg body wt of potassium iodide was administered orally to the animals taking potassium iodide at least 4hours before the respective extracts were administered to ensure iodine had been absorbed into the plasma(this is to ensure an iodine loaded state). All these were administered using orogastric tubes. This is same as incorporating it into the diet fed to the animals, but on account of likely nutrient-chemical interaction that may affect bioavailability, oral solution of potassium iodide was preferred.

Sacrifice of animals

Twenty-four hours after the last administration, the animals were removed, placed in a dessicator glass jar, anaesthetized in chloroform vapour and dissected.

Collection of samples for studies

Whole blood sample obtained by cardiac puncture from each animal was collected into a sterile tube. The whole blood sample collected was allowed to stand for one hour to clot and serum was neatly separated from the clot by a gentle tap with syringe and needle down the side of the tubes. The serum obtained was further subjected to centrifugation using an MSE-table top centrifuge (Minor, England) set at 8000 revolutions per minute (rpm) for 15 minutes, and a clear serum devoid of any trace of hemoglobin obtained. The serum sample obtained from the animals was used for assays.

The thyroid hormones were estimated using enzyme immunoassay system for quantitative determination of these hormones obtained from Microwell (Syntron Bioresearch, Inc. 2774 Loker Avenue West. Carlsbad, CA 92008. USA.

Estimation of thyroxine (T_4)

The microwell T_4 EIA is a solid-phase enzyme immunoassay utilizing the competitive binding principle. Thyroxine in the serum competes with the enzyme-labeled T_4 conjugate, containing 8-Anilino-1-Naphthaloene sulfonic (ANSA) for binding with anti- T_4 antibody immobilized on the microwell surface.

The amount of conjugate that binds to the microwell surface will decrease in proportion to the concentration of T_4 in the serum.

Estimation of triiodothyronine (T_3)

The microwell T_3 EIA is a solid-phase enzyme immunoassay utilizing the competitive binding principle. T_3 presented in the serum sample will compete with

enzyme-labeled T₃ conjugate containing 8-Anilino-1- naphthalene sulfonic acid (ANSA) for binding with antibody immobilized on the microwell surface. The amount of conjugate that binds to the microwell surface will decrease in proportion to the concentration of T₃ in the serum.

STATISTICAL ANALYSIS: Results of all the studies were expressed as mean± standard deviation. Data between groups were analysed using SPSS 2003(version 13).

RESULTS

Effect of onion and garlic extract on iodine incorporation in thyroid hormones

The effect of the onion and garlic extracts on the iodine metabolism of the experimental animals are presented on Table 1.

The effect of the onion and garlic extracts on the iodine metabolism of the experimental animals was studied by determining the thyroid hormone profile.

The mean values of triiodothyronine (T₃) for the experimental animals ranged between 1.23 ± 0.09 ng/ml for the control group and 0.97 ± 0.13 ng/ml for high dose garlic group loaded with iodine. Loading the animals with iodine generally lowered the level of T₃ in their serum. There was a statistically significant ($P < 0.05$) decrease in the level of T₃ in all the iodine loaded groups when compared normal rats. Administration of onion and garlic extract led to a further statistically significant ($P < 0.05$) decrease in the T₃ levels in both normal and iodine loaded rats when compared with the control.

The mean values of thyroxine (T₄) for the experimental animals ranged between 1.19 ± 0.12 IU/ml and 0.85 ± 0.16 IU/ml for the low dose onion normal rats and low dose

garlic group iodine treated respectively. There was generally a statistically significant ($P<0.05$) decrease in T_4 compared to the control group in the experimental animals except for the high dose garlic groups. Oral administration of high dose onion and garlic extract led to a statistically significant ($P<0.05$) dose dependent decrease in the T_4 levels in the normal rats when compared with the control. Although when compared with the control, there were statistically significant ($P<0.05$) falls in the T_4 levels of the animals loaded with iodine these changes were not dose dependent.

Table 1:Effect of oral administration of onion and garlic extracts on thyroid hormones two (T_3 , T_4) of normal and iodine treated wistar rats.

Parameters	T_3 (ng/ml)	T_4 (UI/ml)
Control (Placebo)	1.23± 0.09	1.14± 0.27
LDG	1.08 ± 0.33	1.01 ± 0.20
HDG	1.13 ± 0.14	0.95 ± 0.18
LDO	1.22 ± 0.29	1.19 ± 0.12
HDO	1.07 ± 0.21	1.00 ± 0.26
KI treated (positive control)	1.11 ± 0.14	1.13± 0.27
KI Treated + LDG	1.02 ± 0.11	0.85 ± 0.16
KI treated + HDG	0.97 ± 0.13	1.01 ± 0.08
KI treated + LDO	0.98 ± 0.26	1.18 ± 0.27
KI treated + HDO	1.12 ± 0.29	0.91 ± 0.30

Values are mean ± SD

ND = Not detected.

LDG= Low dose garlic

HDG= High dose garlic

LDO= Low dose Onion

HDO= High Dose Onion

KI =Potassium Iodide

DISCUSSION

The thyroid hormones T_3 and T_4 are unique in that iodine (as iodide) is an essential component of both. In most parts of the world, iodine is a scarce component of soil, and for that reason there is little in food. The role of dietary iodine deficiency as the principal environmental determinant in the development of endemic goitre and other iodine deficiency disorders (IDD) is firmly established. However, there is epidemiological and experimental evidence that concurrent exposure to naturally-occurring thyroid antimetabolites magnifies the goitre endemia and may also affect the expression of the other related disorders (Ubom,1991). Another evidence is the fact that supplementation does not always result in complete eradication of goitre; there have been some well defined instances of geographical pockets of persistence where the condition remains endemic (FAO, 2007). Naturally occurring goitrogens found in many plants are hydrolysed to ultimate goitrogens which mostly inhibit the biosynthesis of thyroid hormones by blocking the coupling of iodotyrosine and iodination of monoiodotyrosine. Some of these goitrogens include sulphurated organic compounds such as sodium thiocyanate, NaSCN, thioglucosides, cyanogenic glucosides and aliphatic disulphides known to be present in *Alliums* (onion and garlic inclusive).

The oral administration of onion and garlic extract brought about a significant depression in the level of triiodothyronine (T_3) and thyroxine (T_4) in the experimental animals which was further depressed due to treatment with iodine. Based on the information in the literature, this is not out of place, as earlier suspected, the presence of

the aliphatic disulphides known to be present in these *Alliums* must have interfered with the oxidation and subsequent incorporation of the iodine present. The thyroid is the only tissue that can oxidize I^- to a higher valence state, an obligatory step in I^- organification and thyroid hormone biosynthesis. This step involves a heme-containing peroxidase and occurs at the luminal surface of the follicular cell. Thyroperoxidase, a tetrameric protein with a molecular mass of 60 kDa, requires hydrogen peroxide as an oxidizing agent. The H_2O_2 is produced by an NADPH-dependent enzyme resembling cytochrome *c* reductase. A number of compounds inhibit I^- oxidation and therefore its subsequent incorporation into MIT and DIT. The most important of these are the thiourea drugs (Murray *et al*, 2003). They are used as antithyroid drugs because of their ability to inhibit thyroid hormone biosynthesis at this step. *Alliums* are known to contain chemical group, disulphides, which are similar in that they also possess the sulphur group to the thio group present in the thiourea drugs (Okoye, 1992). These groups probably act through a similar mechanism to displace the iodide ions present in the thyroid follicle thereby stalling iodination. Thus, leading to a depression in the serum levels of T_3 and T_4 . The depressions in the level of T_3 and T_4 were also dose dependent with the garlic extract exerting a more pronounced effect than the onion extract.

Administration of iodide to experimental rats has been shown to cause transient inhibition of intrathyroid organification of iodine and reduces hormone synthesis (SCF, 2002). This could explain the depression in the level T_3 and T_4 levels in the iodine treated group. Escape from this effect occurs through reduction in iodide transport mechanism until intrathyroid concentration of iodide is below the level necessary to maintain biosynthesis inhibition.

CONCLUSION Onion and garlic are consumed all over the world as spices both by the rich and the poor alike. It is an established fact that dietary factors produce profound changes in body composition, physiology and biochemical functions (Forsythe *et al*, 1986; Erdman and Fordyce, 1989; Carroll, 1991). These two *Alliums* induced significant changes in iodide metabolism in the system. It has always been known or suspected that sulphurated organic compounds e.g. aliphatic disulphide, phenol compounds e.g. flavonoids or polyhydroxyphenols are responsible for the exaggeration or persistence of goitre (Okoye, 1992). These compounds have been shown to be present in onions and garlic. The oral administration of onion and garlic led to a significant depression, in the serum levels of the thyroid hormones, T₃ and T₄. This depression could have been made possible by the competitive displacement of iodide ions in the thyroid follicle by the sulphide derivatives present in onion and garlic. Supplementation with iodine did not improve the level serum thyroid hormones but further led to more depression.

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